CONTRIBUTIONS TO OUR KNOWLEDGE OF SOIL-FERTILITY.

vi. *The Inactivity of the Soil-Protozoa.*

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In papers i., iv., and v. of this series, I have shown that soils contain bacteriotoxins, and fatty substances collectively named agricere. These are affected differently by heat and by the volatile antiseptics. Heat destroys the bacteriotoxins more or less, the extent of the destruction depending naturally upon the temperature and the exposure, but there is an interference through the production of certain toxins developed in some unknown manner during the heating. While, therefore, a moderate heat destroys the natural bacteriotoxins that are in the soil, a higher temperature, or possibly a longer exposure, produces toxins that were not originally present. The volatile disinfectants, on the other hand, have no direct action upon the bacteriotoxins. They act upon the agricere, carrying it to the surface, where it is irregularly deposited upon the angular fragments of soil. Indirectly, the nutrients are rendered more accessible to the solvent action of soil-water, and especially to the attacks of the bacteria, while the bacteriotoxins are more easily dissolved, and, therefore, more easily decay.

By their protozoal hypothesis, Russell and others claim that the soil-bacteria are prevented from multiplying freely by such soil-protozoa as the ciliates (among which *Colpoda cucullus* is very active), and as the soil-amœbæ. The action of heat and of the volatile disinfectants, according to these authors, is chiefly to destroy the protozoal phagocytes, other agencies having only a slight effect.

In the present paper, I have endeavoured to test the action of the soil-phagocytes by adding them purposely to soil, and by using the extracts of raw soil, as was done by Russell and Hutchinson.

But I have taken care to use soil that had not been overheated, and to have controls of unfiltered soil-extracts to compare with the filtered, presumably protozoa-free, extracts. Taken as a whole, my experiments show that Russell's contention cannot be sustained; the protozoa have little or no action in limiting the number of soil-bacteria. This is in agreement with Fred,* who, in one experiment upon the nitrification of compost-soil, found a slight gain in the test which had been treated with ether; he ascribed this to the stimulating effect of the disinfectant, rather than to the destruction of phagocytes.

The action of the soil-bacteriotoxins has been little investigated, although their effect has been known for a considerable time. Martin, for example, found that typhoid bacteria disappeared from raw soil in two days, but persisted for over a year in sterilised soil. Although ascribed to the competition of other bacteria, there can be little doubt that the typhoid bacteria were destroyed by the bacteriotoxins in the raw soil. When some kinds of actively-growing bacteria are added to soil, they rapidly increase, and then die down. For example, *Bac. prodigiosus,* is at the height of its growth betwen the second and third day at 28°; after that, the numbers rapidly fall away. In experiments with soil bacteria, the height of the rise is generally greatest at a later period, on account of the smaller number of bacteria at the start, and also on account of the slower growth of the natural microbes. In plate-cultures, the toxic influence of bacteria, such as *Bact. putidum,* is readily seen, for when many are present upon a plate, few colonies of other bacteria develop. Thus it comes about that the weaker dilutions show a greater number of bacteria than the stronger, for, in the presence of relatively fewer colonies of *Bact. putidum,* the other bacteria are not inhibited. The actual diminution of bacteria, by the products of *Bac. prodigiosus,* was numerically shown in a previous paper.†

The spore-producing soil-bacteria, such as *Bac. vulgatus, Bac. subtilis,* and *Bac. mycoides,* which resist the action of the volatile

* Centralb. f. Bakt., 2G Abt., xxxi., 233.

† These Proceedings, 1911, p.686.

disinfectants, appear to be little influenced by their own bacteriotoxins, but are affected by the toxins of other bacteria. This indifference was noted by Russell and Hutchinson, and although they claim that no bacteriotoxins are present in soil, they speak of the inhibiting action of bacteria added in the course of their experimental work.

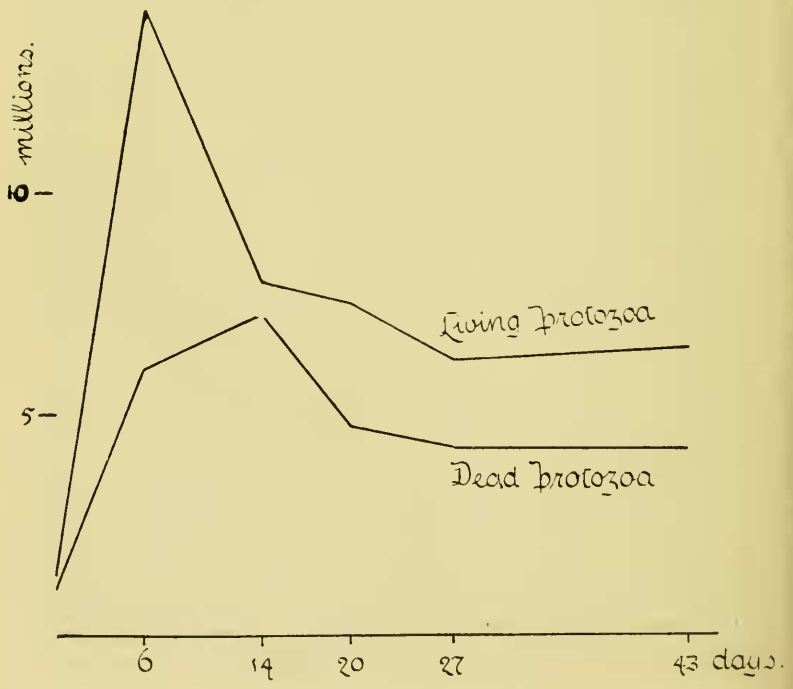
These authors had tested the effect of the protozoa indirectly by comparing the growth of bacteria in raw soils, or in treated soils to which raw soil had been added, with the growth of bacteria contained in suspensions of raw soil freed from protozoa by filtration through cotton wool. The effect of filtered and unfiltered suspensions was not tested, although this appears to be the more reasonable method of testing the matter. Their experiments with heated soils are of little value, for two reasons. First, the temperature and period of exposure were excessive for the object in view, namely, the destruction of the protozoa; and, secondly, they ignored the effect of the bacteriotoxins and heat-toxins. As it appeared that a confirmation of their work was necessary, certain experiments were begun with this object.

In the first, a good arable soil was treated, for two days, with 2 per cent. of chloroform, and 20 grm. portions were weighed into small, wide-necked, ounce-bottles. These were divided into two sets. Each portion of one set received four c.c. of a suspension of a ciliate, *Colpoda cucullus*, while the portions of the other set were treated with the same quantity of the same suspension after it had been heated at 65° for ten minutes. Thus, one set received a suspension of living, the other of dead protozoa. The ciliates had been grown in 4 per cent. bean-infusion, and had been derived from a garden-soil. It was not a pure culture, and had been partly washed in 0·2 per cent. saline, but as this caused an encysting of the ciliates, the washing could not be continued until the great bulk of the bacteria had been eliminated. As it was, each portion received 400 motile forms of *Colpoda cucullus*, besides many bacteria and encysted ciliates. The bottles were covered with a small bell-jar, and incubated at 28°. In this, and the succeeding two experiments, the soils, containing at the start 20·9 per cent. of moisture,

slowly dried; the figures obtained after the forty-fourth day are, therefore, not recorded. In the later experiments, the bottles were weighed, and the loss of water made up from time to time. In the last experiments, the loss of moisture was avoided by using corks fitted with a short piece of glass-tubing, terminated with an open point of about 1 mm. bore.

EXPERIMENT i.—THE ADDITION OF PROTOZOA.

Chloroformed soil.	Bacteria grown at 28° in millions per grm.					
	Start.	6 days.	14 days.	20 days.	27 days.	43 days.
Living protozoa ...	1·3	14·3	8	7·5	6·2	6·5
Dead protozoa ...	1·0	6	7·3	4·7	4·3	4·3



Although living protozoa were added to the soils of the first set, the bacteria increased enormously in six days. A phagocytic activity is not apparent. The subsequent decline may, however, be due to the ciliates, but it is more likely caused by the secretion of toxins by the bacteria. The continued excess of bacteria, in the test containing the living protozoa, in no way confirms the phagocytic hypothesis. Indeed, it is evident that the reason for the rapid increase of the numbers, in the first test, was caused by the introduction of a number of rapidly growing, feebly-resistant bacteria, which soon succumbed to the effect of their own toxin. The resistant bacteria, being also more numerous, probably account for the continuation of the greater number, as time went on. The nature of the colonies upon the plates was instructive. Those derived from the "living" soils were chiefly of the translucent-white or yellowish glistening kind, characteristic of the *coli-fluorescens* group of bacteria, while those from the "dead" soils were mostly opaque, white and granular, indicative of the *subtilis-vulgatus* bacilli. The odour of the plates was also marked. Those of the "living" set had a disagreeable, putrefactive smell, in sharp contrast with the faint odour of the other. By the twenty-seventh day, the distinctive odours had disappeared, and the colonies were very much the same in both tests.

The suspensions had been tested for living protozoa by infecting sterile 4% bean-infusions. The heated suspension contained none, while the raw suspension gave rise to many. On the fourteenth day, the soils were tested in a similar manner. Both contained Colpoda, and the "living" soil contained amœbæ in addition. Upon testing the original chloroformed soil, it was discovered that the protozoa were still alive; a luxuriant growth of *Colpoda cucullus* being obtained. Thus the treatment with 2% chloroform had not been sufficient to destroy the encysted ciliates.

For destroying protozoa, Russell and Golding used 2% of carbon bisulphide or toluol, allowing it to act for two days; while, for field-work, they suggest the employment of from two or three cwt. per acre, as a suitable quantity. This is, roughly, the equivalent of from 0·01 % to 0·02 %. Russell and Hutchinson used 4 % of

toluene to kill off all protozoa, but, in the later part of their paper, the statement occurs that toluene does not kill off all the larger organisms, one, at least, a small ciliated protozoon being left; and this is probably concerned with the diminution of the activity of the treated soil, after a long period, as, for example, in the second crop. The impression is left, however, that the disinfectant kills off living and encysted forms of *Colpoda cucullus*, the chief food of which is said to be bacteria.

In my experiments, I found that *Colpoda cucullus* was, of all the soil-protozoa, least affected by disinfectants. It occurred in infusions seeded with soils which had been treated for three days with 20% of toluene (Kahlbaum), or with 10% of chloroform (Schering).

With regard to its food, the partiality for bacteria is open to question. From observation, they appear to feed upon organic débris of any kind, and any bacteria that they consume are drawn in accidentally. They are specially fond of the slimy matter exuded by the encysting cell. It should not be forgotten that the digestion of the organic débris will give rise to waste products containing nutrients available for bacteria, thus augmenting the food at the disposal of the remaining microbes, which will respond by growing more quickly. The bacterial increase should not, for this reason, be lessened by the presence of Colpoda. It appears that, if any real phagocytic effect in reducing the bacterial numbers is to be ascribed to any protozoa, it should be to the amœbæ rather than to the ciliates. The amœbæ are destroyed by comparatively small amounts of disinfectants; they were detected in infusions seeded with soils which had been treated with 1% of chloroform, but not with 2%.

It is unfortunate that Russell and Hutchinson did not use enough disinfectant to ensure the complete destruction of all the protozoa in their experimental work, as there is the doubt raised that, so far as the protozoa are concerned, their disinfection had been abortive. And yet the point claimed by these authors is, that the protozoa, and especially Colpoda, had been destroyed, and, in consequence, the bacteria had increased. Might not the proportion

of protozoa that had been destroyed, have been proportional to the bacteria that were killed; and that, so far as numbers are concerned, the *status quo* remained after the treatment with disinfectants?

From the appearance of the protoplasm and the absence of food-granules, Goodey* concludes that the Colpoda first to appear in soil-cultures, have emerged from the encysted condition, and that they, therefore, do not functionate as a factor in limiting the bacterial activity in soils.

As the ciliates, such as *Colpoda cucullus,* cannot be credited with the limitation of the soil-bacteria, we must examine the claims of the amœbæ; and be it remembered, that we are not so much concerned with phagocytosis as with the limitation of the bacteria.

Even if the amœbæ do actively ingest bacteria, in the soil, there is no evidence that the net result may not be an increase of the residual microbes from the stimulating influence of the excreted products of the digested bacillary protoplasm.† On the other hand, it is possible that substances of the nature of immune bodies may be secreted or excreted by the amœbæ. The matter clearly cannot be decided *ex cathedra,* and, accordingly, an experiment was begun, in which a number of amœbæ were added to a soil that had been freed from protozoa by heating at 65° and treatment with chloroform. Subsequent tests showed that the soil was free from protozoa. A suspension of amœbæ, *Amoeba limax,* from a

* Proc. Roy. Soc. B.84, 18/8/11, p.179.

† The amœbæ undoubtedly are phagocytes, but they certainly do not englobe every microbe they chance to meet, for I have watched soil-amœbæ moving in plant-infusions, and in no case have I seen the undoubted ingesting of a bacterium. I have seen the protruding pseudopodia push aside the living bacteria, and pass over the dead microbes [a trace of methylene blue added to the drop under examination colours the dead cells but not the living] which can be traced under the amœba as it glides along, and which are left upon the spots they originally occupied. A motile bacterium may touch the protozoon, and dart off again, or it may be caught, presumably by the flagella, and after wriggling about for some seconds, swim away. Again, a protruding pseudopodium may touch a bacterium and immediately retract, or a distinct angular bay may be formed as the pseudopodium meets and passes the microbe. Rotating

garden soil, had been seeded into a bean-infusion, and, after a time, a rich growth of amœbæ was found. The suspension was centrifugalised, and the sediment rapidly washed with 0·2% saline. The amœbæ were suspended in saline, a part of which was heated for 10 minutes at 62° to 64°. To each 20 grm. portion of soil, 4 c.c. of suspension were added. This contained, in the case of the unheated suspension, 6,080 living motile amœbæ, no motile ciliates, many cysts, presumably of the latter, and many bacteria.

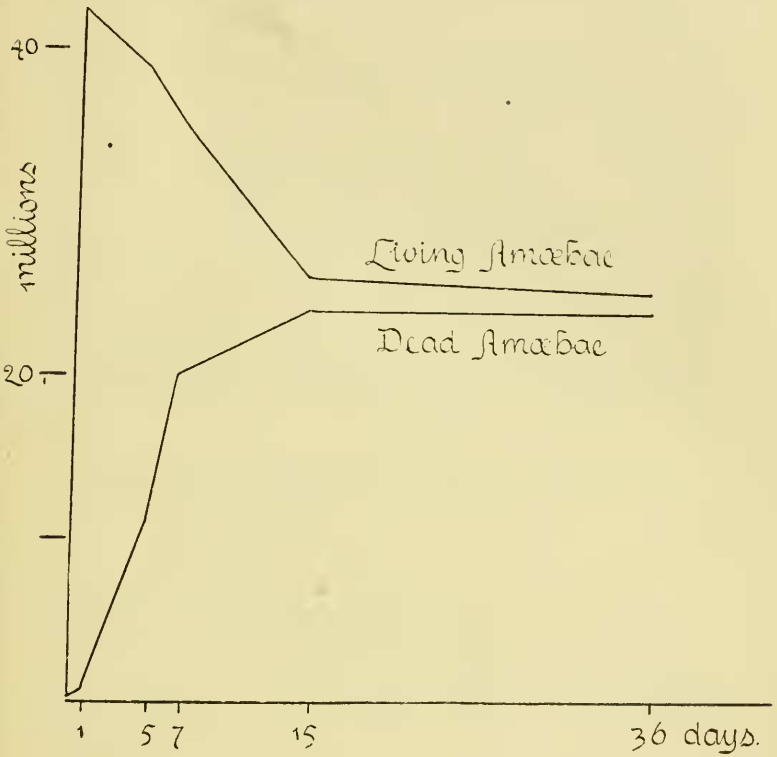
EXPERIMENT ii.—THE ADDITION OF AMŒBÆ.

Heated soil.	Bacteria grown at 28° in millions per grm.					
	Start	1 day	5 days	7 days	15 days	36 days
Living amœbæ ...	0·3	42·5	39	35	26	25
Dead amœbæ ...	0·2	0·9	11	20	24	24

The experiment was repeated and confirmed at a later date, with a poor sandy soil. The tests were contained in small bottles closed with wooden corks, through which passed glass tubes furnished with open capillary ends of approximately 1 mm. bore. The evaporation from the soil was very small, the loss being equal to only 0·37% in 73 days at 22°. Each test received four c.c. of a suspension of *Amœba limax* in 0·2% saline. This quantity contained 2,720 moving and 800 encysted amœbæ, together with a mixed

pairs of bacteria cease their motion as the protozoal arm touches them, but in a few seconds they are as active as ever. I have seen an amœba, in moving forwards, touch a rod-shaped microbe which adhered to the surface, sliding over but still maintaining its position in the field, until the terminal was reached. There it remained attached. Meanwhile, another microbe was similarly treated, but somehow became detached from the terminal. In its circumambient wandering, the amœba touched the same bacterium, and both became fixed to the terminal tuft of barely visible slime, until a fragment of débris, encountered by the protozoon, proved too weighty, and fragment and bacteria were left behind. Mouton [Ann. de l'Inst. Past., xvi., p.476] seems to have seen, in this entanglement, an agglutination of the microbes by an agglutinin secreted by the pulsating vacuole.

bacterial flora, but with no other protozoa. Half of the suspension was heated at 65° for 30 minutes. The soil had been treated with 10% of chloroform to destroy any native amœbæ.



Experiment iiα.—The Addition of Amœbæ.

	Bacteria grown at 22° in millions per grm.						
	Start	5 days	14 days	21 days	33 days	45 days	73 days
Living amœbæ ...	3·0	44·0	8·4	3·8	3 1	1·0	5 2
Dead amœbæ ...	0·8	5·9	2·1	3·9	1·4	0·4	1·2

The bacteria in the soils, seeded with living amœbæ and bacteria, multiplied very rapidly during the first day. This was due to the quickly growing nature of the added microbes, which, from the examination of the colonies upon the plates, were seen to be of the *coli-fluorescens* type, and, among them, *Bact. putidum* was prominent. The decline in the numbers may have been caused by the phagocytic propensities of the amœbæ, but it was more probably the result of the action of the bacteriotoxins secreted by the bacteria themselves. In this, as in the first experiment, there is no evidence of any rapid increase in the amœba-free soil.

A general observation of the behaviour of the bacteria in soils, leads one to believe that the kinds resistant to heat and disinfectants, are little influenced either by their own toxins or by those of other groups. Such, however, does not appear to strictly hold, for their growth is certainly restricted by the presence of toxins of other groups, as the following approximate count of the rough, opaque colonies upon the plates, shows.

EXPERIMENT ii.—BACTERIA OF THE *Subtilis-vulgatus* TYPE.

	In millions per grm.					
	At start	1 day	5 days	7 days	15 days	36 days
Unheated suspension ...	0·13	0·50	10	7	12	10
Heated suspension ...	0·13	0·53	11	15	18	17

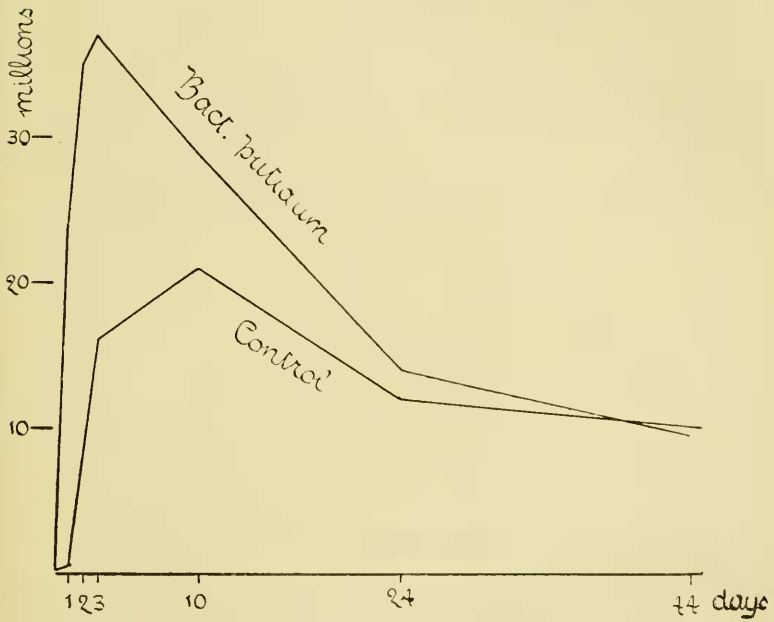
On comparing the numbers with those of the total bacteria, it is seen that the non-resistant have a decided inhibiting action upon the resistant bacteria, and, although the latter increase as time goes on, their multiplication is not so rapid in the presence as in the absence of the toxins of the less resistant and more rapidly growing forms.

Upon noting that *Bact. putidum* was one of the chief bacteria in the unheated suspension, a series of portions of soil were seeded with a pure culture of this organism, and, for the purpose of con-

trol, a second series received water. The soil had been treated with toluene, and had been heated to 65°.

EXPERIMENT iii.—THE ADDITION OF *Bact. putidum*.

Heated and toluened soil.	Bacteria grown at 28° in millions per grm.						
	Start	1 day	2 days	3 days	10 days	24 days	44 days
Bacteria	0·098	24	35	37	29	14	9·5
Control	0·095	0·3	9·4	16	21	12	10



The experiment shows the same rapid rise following the addition of one of the components of the suspension used in Expt. ii., and the control is similar in its behaviour to the heated suspension series. As a whole, the experiment tells us that the same general

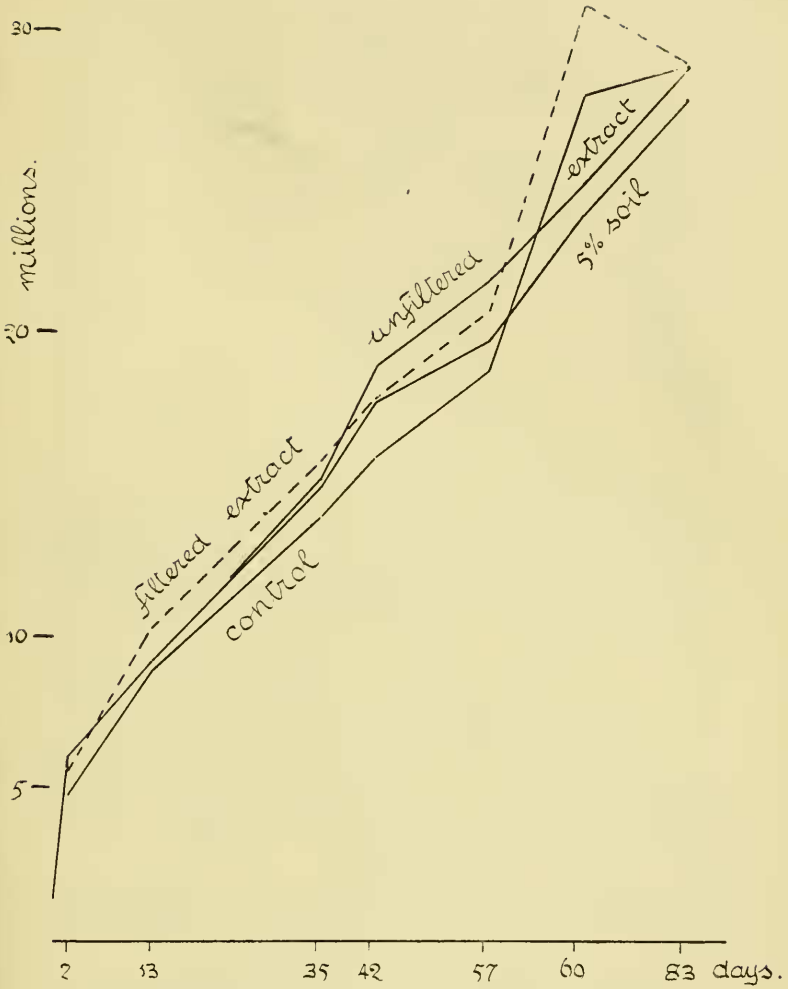
multiplication of bacteria occurs in the presence, as in the absence of amœbæ.

In the apparent absence of protozoal activity in these experiments, it seemed necessary to confirm some of Russell and Hutchinson's results. The most telling of their experiments was one in which, as the result of adding a filtered suspension to a toluened soil, the bacteria rose from 66 millions on the 20th, to 166 millions on the sixtieth day. No test was, however, made with the unfiltered as against the filtered suspension. The experiment was not confirmed, and, as it is possible that the results might have been abnormal, a repetition of a certain portion of it was decided upon.

An alluvial soil was air-dried, and treated for two days with 5% chloroform. After the evaporation of the solvent, a number of 20 grm. portions were weighed out into small bottles, and moistened with 4 c.c. of water or extract, a proportional quantity of water being added to the tests which received the gram of air-dried, untreated soil. The amount of water lost by evaporation was calculated weekly or biweekly from the loss of weight of eight bottles, two from each set, and the loss was made good. The moisture in the soils varied up and down from 19·6 to 15·4. The extract was made by shaking 100 grm. of soil with 500 c.c. of water for 20 minutes, and filtering half of it through five inches of tightly packed, cotton wool. This removed the larger protozoa, such as *Colpoda cucullus*, but the cysts of smaller ciliates were not retained, as was shown by their growth in bean-infusion. The experiment was made in duplicate, one set being incubated at 28°, the other at 15°.

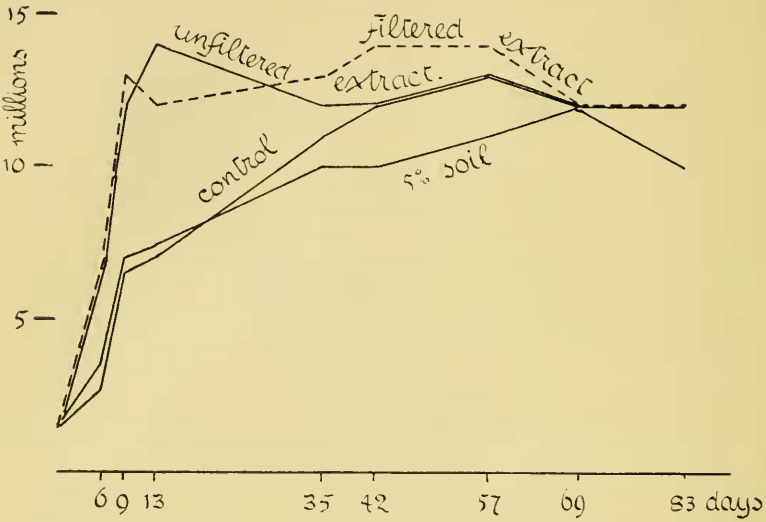
EXPERIMENT iv., *a*—THE ADDITION OF SOIL AND ITS EXTRACTS.

Chloroformed soil (alluvial).	Bacteria grown at 28° in millions per grm.								
	Start	2 d.	6 d.	13 d.	35 d.	42 d.	57 d.	69 d.	83 d.
1, Water control ...	1·33	4·8	4·9	9·0	14	16	19	28	29
2, Unfiltered extract.	1·36	6·0	6·3	9·2	15·3	19	22	25	29
3, Filtered extract ...	1·34	5·7	6·4	10·2	15·8	18	21	31	29
4, 5% untreated soil..	1·40	5·7	5·3	9·2	15	18	20	24	28



EXPERIMENT iv.,*b*—THE ADDITION OF SOIL AND ITS EXTRACTS.

Chloroformed soil (alluvial)	Bacteria grown at 15° in millions per grm.								
	Start	6 d.	9 d.	13 d.	35 d.	42 d.	57 d.	69 d.	83 d.
1, Water control ...	1·33	2·7	6·5	7	11	12	13	12	12
2, Unfiltered extract.	1·36	6·9	12	14	12	12	13	12	12
3, Filtered extract ...	1·34	6·7	13	12	13	14	14	12	12
4, 5% untreated soil...	1·40	3·5	7	7·5	10	10	11	12	10



These soils had not been heated, and do not show the rapid rise that occurred in the earlier experiments. The curves of iv. *a*, for the most part, rise fairly steadily, and there is little difference between them. We see no indication of any influence having been exerted by phagocytic protozoa derived either from the unfiltered extract or from the untreated soil. Beyond the fact that Nos. 2, 3, and 4 received originally more bacteria than the control, and consequently obtained a lead, these tests practically give the same result. The protozoa cannot be said to have any action upon the soil-bacteria at 28°, at which temperature they are very active.*

The curves of the tests at 15° differ from those at 28°. Those which received the extracts, gave a more rapid bacterial growth within the first ten days, but, as at the higher temperature, there is no pronounced evidence of protozoal activity.

One of the points brought out, is the influence of temperature upon bacterial growth. At 15° the numbers never rose above 15 millions, and remained constant between 10 and 15 millions per

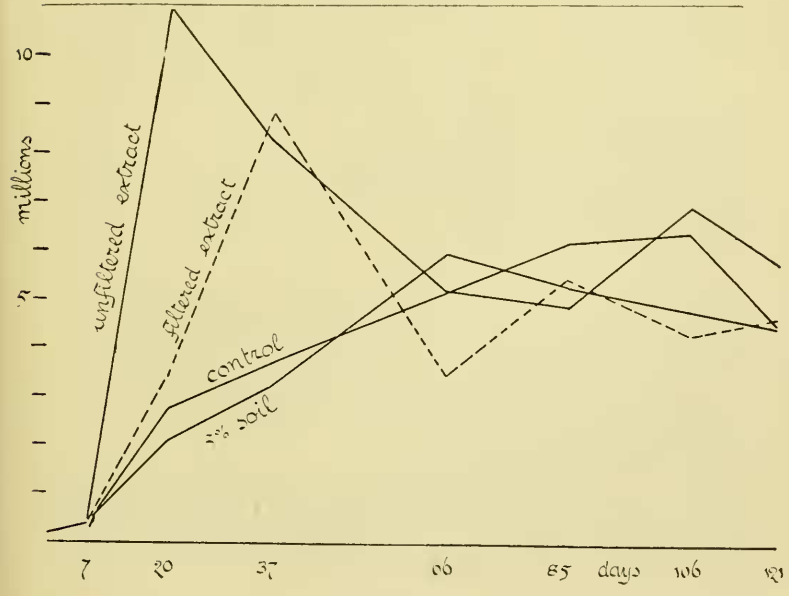
* The period of multiplication for *Amœba limax* was found to be $1\frac{3}{4}$ hours at 37°, 8 hours at 28°, and 28 hours at 15°.

grm. At 28°, the rise was steady, and by the eightieth day, the numbers lay between 25 and 30 millions.

A variation of the preceding experiment was made by using a poor sandy soil, and incubating the tests at 22°. The soil, which contained *Amœba limax*, was treated with 4% of chloroform for two days, and the moisture was brought up to 17%. As, however, a strong growth of moulds developed upon the surfaces of the soils in the tests, the moisture was, by the thirty-seventh day, allowed to fall to 13%, at which it was maintained.

Experiment v.—The Addition of Soil and its Extracts.

Chloroformed soil (poor sandy).	Bacteria grown at 22° in millions per grm.							
	Start	7 d.	20 d.	37 d.	66 d.	85 d.	106 d.	121 d.
1, Water control	0·14	0·3	2·8	3·7	(0·2)	6·2	6·4	4 4
2, Unfiltered extract ...	0·17	0·5	11·0	8·3	5·2	4·9	7·0	5·8
3, Filtered extract ..	0·15	0·5	3·4	8·9	3·5	5·5	4·3	4·7
4, 5% untreated soil ..	0·17	0·5	2·1	3·2	6·0	5·3	(1·6)	4·5



The experiment generally confirms the previous ones, and shows that the removal of some of the protozoa, by filtering the soil-extract through cotton wool, has little influence upon the multiplication of the bacteria in the soil, beyond what is to be expected from the behaviour of the microbes in the extracts.

The curves of the last two experiments do not show the sharp rise noted in the experiments with protozoa, etc., in the earlier part of this paper. There is little doubt that the rapid rise was occasioned by the destruction of the soil-toxins by heat, and, in a confirmatory experiment, the soil was heated to show that such was actually the case. The same alluvial soil was used as in experiment iv. *a*, and the incubation temperature was the same, viz. 28°. It was heated at 60° to 70° for half-an-hour, but otherwise the conditions were the same. A fifth test was included to show the effect of chloroform.

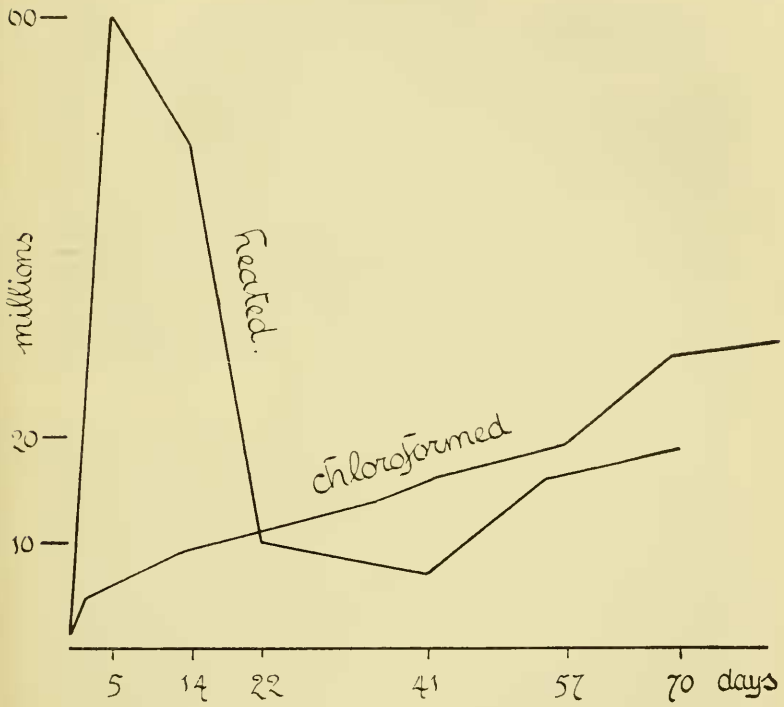
Experiment vi.—The Use of Heated Soil.

Heated soil (alluvial).	Bacteria grown at 28° in millions per grm.						
	Start.	5 dys	14 dys	22 dys	41 dys	55 dys	70 dys
Chloroformed							
1, Water control ...	1·24	60	48	19	7	16	19
2, Unfiltered extract ...	1·27	56	47	12	9	15	19
3, Filtered extract ...	1·25	55	47	15	8	16	17
4, 5% untreated soil ...	1·54	65	43	17	14	10	17
Not chloroformed							
5, Water control ...	1·22	51	54	14	7	13	15

The numbers are so near to one another, especially with Nos. 2 and 3, which received the unfiltered and filtered soil-extracts, that the inactivity of the soil-protozoa is well demonstrated. The results with heated soils have confirmed those obtained in the previous experiments with unheated soils.

The effect of heat alone, and of chloroform alone, is very marked, as can be seen by comparing the curves of the control tests in Experiments iv., *a*, and vi. These tests were made upon the same soil, containing the same amount of moisture, and were incubated

at the same temperature. I believe the moisture and temperature are of more importance in modifying the bacterial content of soils than one would imagine, and experiments concerning these influences are in progress. In heated soils, the bacteria grow very rapidly at first, then, as the toxin accumulates, the numbers fall almost as sharply, after which they slowly rise. With chloroform alone, the numbers increase slowly and steadily, as if nutrients were being slowly utilised.



The extracts with which the soils had been treated, in these experiments, contained not only protozoa and bacteria, but also nutrients and toxins, as I have already shown in the first paper of this series. As it is just possible that these two latter substances might have a certain, though small, influence in increasing or

decreasing the numbers of bacteria, an experiment was made to test the matter. The same alluvial soil, after chloroforming, received four c.c. of water, and of porcelain-filtered extracts of the strength used, viz., 100 grm. to 500 c.c. After filtering, a portion was boiled for an hour under an aërial condenser, and cooled. The moisture-content of the tests was 19·1%.

EXPERIMENT vii.—THE EFFECT OF PORCELAIN-FILTERED EXTRACTS.

Alluvial soil (chloroformed).	Bacteria grown at 15%, in millions per grm.				
	At start.	2 days.	7 days.	14 days.	36 days.
Water control	0·5	2·5	6·7	9·8	10·2
Filtered extract	0·5	2·3	5·7	8·5	11·6
Filtered extract, heated ..	0·5	2·6	8·7	12·0	12·4

The unheated filtered extract had, at first, a toxic action when the numbers were lower than the control. But, as the added toxin decayed, the numbers rose. The heated extract had a pronounced nutritive effect. The differences are not great, but they indicate that the soluble substances in the extracts have a certain, though small, influence upon the growth of bacteria.

From the foregoing, it will have been seen that the larger ciliates, as *Colpoda cucullus*, are not destroyed when comparatively large amounts of volatile disinfectant are added to soil. Upon adding suspensions of protozoa, there was no evidence of any limitation in the numbers of the soil-bacteria. Any enhanced effect was due to the addition of the bacteria contained in the suspensions. The filtration of a soil-extract had no influence, beyond that of removing some of the bacteria in the suspension. Any phagocytic tendencies that the soil-protozoa possess, have no influence in limiting the numbers of bacteria in the soil. So far as the growth of bacteria is concerned, the effect of heat is of a different character from that of a volatile disinfectant. Inferentially, the toxins and nutrients of the soil are alone concerned with the changes that occur when soils are heated, or treated with volatile disinfectants.